

bc
CORRECTED
VERSION *

PCT

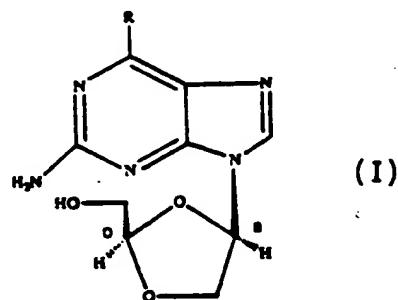
WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : A61K 31/52, C07D 405/04 C07D 473/00, C07F 5/02	A1	(11) International Publication Number: WO 94/04154 (43) International Publication Date: 3 March 1994 (03.03.94)
(21) International Application Number: PCT/US93/08044		(74) Agents: ZALESKY, Cheryl, K. et al.; Kilpatrick & Cody, 1100 Peachtree Street, Suite 2800, Atlanta, GA 30309 (US).
(22) International Filing Date: 25 August 1993 (25.08.93)		(81) Designated States: AU, CA, JP, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).
(30) Priority data: 07/935,515 25 August 1992 (25.08.92) US		Published <i>With international search report.</i>
(71) Applicants: UNIVERSITY OF GEORGIA RESEARCH FOUNDATION, INC. [US/US]; The University of Georgia, Athens, GA 30602 (US). EMORY UNIVERSITY [US/US]; 1380 S. Oxford Road, Atlanta, GA 30322 (US).		
(72) Inventors: CHU, Chung, K. ; 120 Orchard Knob Lane, Athens, GA 30605 (US). SCHINAZI, Raymond, F. ; 1524 Regency Walk Drive, Decatur, GA 30033 (US).		

(54) Title: ENANTIOMERICALLY PURE β -D-DIOXOLANE-NUCLEOSIDES



(57) Abstract

A method and composition for the treatment of humans infected with HIV that includes the administration of an HIV treatment amount of an enantiomerically pure β -D-dioxolanyl purine nucleoside of formula (I), wherein R is OH, Cl, NH₂, or H, or a pharmaceutically acceptable salt or derivative of the compound, optionally in a pharmaceutically acceptable carrier or diluent.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	FR	France	MR	Mauritania
AU	Australia	GA	Gabon	MW	Malawi
BB	Barbados	GB	United Kingdom	NE	Niger
BE	Belgium	GN	Guinea	NL	Netherlands
BF	Burkina Faso	GR	Greece	NO	Norway
BG	Bulgaria	HU	Hungary	NZ	New Zealand
BJ	Benin	IE	Ireland	PL	Poland
BR	Brazil	IT	Italy	PT	Portugal
BY	Belarus	JP	Japan	RO	Romania
CA	Canada	KP	Democratic People's Republic of Korea	RU	Russian Federation
CF	Central African Republic	KR	Republic of Korea	SD	Sudan
CG	Congo	KZ	Kazakhstan	SE	Sweden
CH	Switzerland	L1	Liechtenstein	SI	Slovenia
CI	Côte d'Ivoire	LK	Sri Lanka	SK	Slovak Republic
CM	Cameroon	LJ	Luxembourg	SN	Senegal
CN	China	LV	Latvia	TD	Chad
CS	Czechoslovakia	MC	Monaco	TG	Togo
CZ	Czech Republic	MG	Madagascar	UA	Ukraine
DE	Germany	ML	Mali	US	United States of America
DK	Denmark	MN	Mongolia	UZ	Uzbekistan
ES	Spain			VN	Viet Nam
FI	Finland				

ENANTIOMERICALLY PURE 8-DIOXOLANE-NUCLEOSIDES

The government has rights in this invention by virtue of grants from the Public Health Service of the National Institute of Allergy and Infectious 5 Diseases, and the Department of Veterans Affairs that partially funded research leading to this invention.

Background of the Invention

This invention is in the area of organic 10 compounds with antiviral activity, and in particular provides a process for the preparation of enantiomerically pure 8-D-dioxolane nucleosides, and methods for the treatment of viral diseases that includes administering an effective amount of 15 one or more of the described compounds.

A number of 2',3'-dideoxynucleosides have been found to be potent antiviral agents against human immunodeficiency virus (HIV), the causative agent of acquired immunodeficiency syndrome (AIDS). AZT 20 (3'-azido-2'-deoxythymidine, Mitsuya, H.; Broder, S. Proc. Natl. Acad. Sci. U.S.A., 1986 83, 1911) was the first compound approved by the U.S. Food and Drug Administration for the treatment of patients with AIDS or AIDS-related complex. Other 25 synthetic nucleosides have now either been approved or are undergoing various stages of clinical trials, including 2',3'-dideoxyinosine (DDI), 2',3'-dideoxycytidine (DDC) (see Yarchoan, R. et al., Science, 1989, 245, 412), and 2'-fluoro-30 arabinofuranosyl-2'-3'-dideoxycytidine (Martin, T.A., et al., J. Med. Chem., 1990, 33, 2137; Watanabe, K.A., et al., J. Med. Chem., 1990, 33, 2145; Sterzycki, R.Z., et al., J. Med. Chem., 1990 33, 2150).

35 After cellular phosphorylation to the 5'-triphosphate by cellular kinases, these synthetic nucleosides may be incorporated into a growing

-2-

strand of viral DNA, causing chain termination due to the absence of the 3'-hydroxyl group.

The stereochemistry of nucleoside derivatives play an important role in their biological activity. The C1' position of the ribose in the nucleoside (the carbon bound to the nitrogen of the heterocyclic base) is a chiral center because the carbon is attached to four different moieties. Likewise, there is an optically active center at C4' of the nucleoside (the ring carbon bound to the hydroxymethyl group that is phosphorylated in nucleotides). In the naturally occurring nucleosides, both the base attached to the C1' and the hydroxymethyl group attached to the C4' atom are in the β -configuration (above the plane of the sugar). The corresponding non-naturally occurring α -isomers (in which the moieties are below the plane of the sugar) are rarely biologically active, and are typically toxic.

An analysis of the solid-state conformations of six active and two inactive anti-HIV nucleoside agents was recently performed to attempt to correlate the presence or absence of certain stereochemical features with high HIV activity.

Van Roey, P., et al., J. Am. Chem. Soc., 1988, 110, 2277; and Van Roey, P., et al., Proc. Natl. Acad. Sci. U.S.A., 1989, 86, 3929.

There has been recent interest in the synthesis of nucleoside derivatives in which the 3'-carbon of the nucleoside has been replaced with a heteroatom. Norbeck, D.W., et al., in Tet. Lett., 1989, 30, 6263, reported the synthesis of (\pm) -1-[(2B,4B)-2-(hydroxymethyl)-4-dioxolanyl]thymine (referred to below as (\pm) -dioxolane-T, see Figure 1), that results in a racemic mixture of diastereomers about the C4' atom. The product is a derivative of 3'-

-3-

deoxythymidine in which the C3' atom has been replaced with an O3' atom. The product was synthesized in five steps from benzyloxyaldehyde dimethylacetal and (\pm)-methyl glycerate to produce 5 a 79% yield of the 1:1 diastereomeric mixture. The X-ray crystallographic analysis of the product revealed that the dioxolane ring adopts the 3T_4 conformation commonly observed in ribonucleosides, with the O3' atom in the endo position. Norbeck 10 reported that the racemic mixture of dioxolane-T exhibits an anti-HIV activity of 20 μ M in ATH8 cells, and attributed the low efficacy against the virus to an effect of the endo conformation of the O3' atom. Tetrahedron Letters 30 (46), 6246, 15 (1989).

European Patent Application Publication No. 0 337 713 and U.S. Patent No. 5,041,449, assigned to IAF BioChem International, Inc., disclose that a generic formula of 2-substituted-4-substituted-1,3-dioxolanes exhibit antiviral activity.

Belleau, et al., in the Fifth International Conf. on AIDS, Montreal, Canada June 4-9, 1990, paper No. T.C.O.1., reported a method of synthesis of cytidine nucleosides that contain oxygen or 25 sulfur in the 3'-position. The dioxolane ring was prepared by the condensation of RCO_2CH_2CHO with glycerin. As with the Norbeck synthesis, the Belleau synthesis results in a racemic mixture of diastereoisomers about the C4' carbon of the 30 nucleoside. Belleau reported that the sulfur analog, referred to as NGBP-21 or (\pm) BCH-189 (see Figure 1), has anti-HIV activity.

European Patent Application No. 92300056.6 to Belleau discloses the use of BCH-189 for the 35 treatment of hepatitis B virus (HBV). BCH-189 is now in clinical trials under the supervision of the U.S. Food and Drug Administration.

-4-

U.S. Patent No. 5,047,407 and European Patent Application Publication No. 0 382 526, also assigned to IAF Biochem International, Inc. disclose that a generic formula of 2-substituted-5-
5 substituted-1,3-oxathiolane nucleosides have antiviral activity.

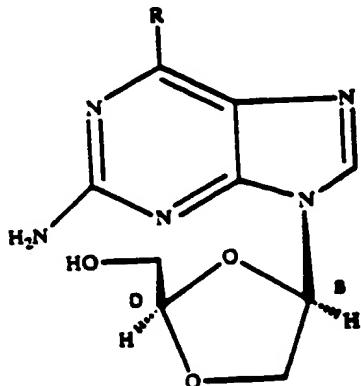
As of the priority date of this application, a method of synthesis of a nucleoside analog with an oxygen in the 3'-position that results in an enantiomerically pure dioxolane nucleoside that has the same stereochemistry as the nucleosides found in nature (the S stereoisomer) has not been reported. There is a need for such a synthesis as a research tool to provide more information on the
10 effect of stereochemistry on the anti-viral activity of nucleoside derivatives, and to provide
15 new anti-HIV agents.

It is therefore an object of the present invention to provide a method of synthesis of enantiomerically pure dioxolane nucleosides.
20

It is another object of the present invention to provide enantiomerically pure dioxolane nucleosides with significant anti-HIV activity.

Summary of the Invention

25 A method for the treatment of humans infected with HIV that includes administering an HIV treatment amount of an enantiomerically pure S-D-dioxolanyl purine nucleoside of the formula:



-5-

wherein R is OH, Cl, NH₂, or H, or a pharmaceutically acceptable salt or derivative of the compound, optionally in a pharmaceutically acceptable carrier or diluent. The compound
5 wherein R is chloro is specifically referred to as (-)-(2R,4R)-2-amino-6-chloro-9-[(2-hydroxymethyl)-1,3-dioxolan-4-yl]purine. The compound wherein R is hydroxy is (-)-(2R,4R)-9-[(2-hydroxymethyl)-1,3-dioxolan-4-yl]guanine. The compound wherein R is
10 amino is (-)-(2R,4R)-2-amino-9-[(2-hydroxymethyl)-1,3-dioxolan-4-yl]adenine. The compound wherein R is hydrogen is (-)-(2R,4R)-2-amino-9-[(2-hydroxymethyl)-1,3-dioxolan-4-yl]purine.

The specifically disclosed 8-D-dioxolane
15 nucleosides, or their pharmaceutically acceptable derivatives or salts or pharmaceutically acceptable formulations containing these compounds are useful in the treatment of HIV infections and other related conditions such as AIDS-related complex (ARC), persistent generalized lymphadenopathy (PGL), AIDS-related neurological conditions, anti-HIV antibody positive and HIV-positive conditions, Kaposi's sarcoma, thrombocytopenia purpura and opportunistic infections. In
20 addition, these compounds or formulations can be used prophylactically to retard the progression of clinical illness in individuals who are anti-HIV antibody or HIV-antigen positive or who have been exposed to HIV.
25

In another embodiment, the invention includes a method for the treatment of humans infected with HIV that includes administering an HIV treatment amount of a prodrug of the specifically disclosed enantiomerically pure 8-D-dioxolanyl purine
30 nucleosides. A prodrug, as used herein, refers to a pharmaceutically acceptable derivative of the

-6-

specifically disclosed nucleoside, that is converted into the nucleoside on administration in vivo. Nonlimiting examples are pharmaceutically acceptable salts (alternatively referred to as

- 5 "physiologically acceptable salts"), and the 5' and N⁶ acylated or alkylated derivatives of the active compound (alternatively referred to as "physiologically or pharmaceutically acceptable derivatives"). In one embodiment, the acyl group
10 is a carboxylic acid ester in which the non-carbonyl moiety of the ester group is selected from straight, branched, or cyclic C₁-C₂₀ alkyl alkoxyalkyl including methoxymethyl; aralkyl including benzyl; aryloxyalkyl such as
15 phenoxyethyl; aryl including phenyl optionally substituted with halogen, C₁ to C₄ alkyl or C₁ to C₄ alkoxy; a dicarboxylic acid such as succinic acid; sulfonate esters such as alkyl or aralkyl sulphonyl including methanesulfonyl; and the mono, di and
20 triphosphate esters.

As used herein, the term alkyl specifically includes but is not limited to methyl, ethyl, propyl, butyl, pentyl, hexyl, isopropyl, isobutyl, sec-butyl, t-butyl, isopentyl, amyl, t-pentyl, cyclopentyl, and cyclohexyl. As used herein, the term acyl specifically includes but is not limited to acetyl, propionyl, butyryl, pentanoyl, 3-methylbutyryl, hydrogen succinate, 3-chlorobenzoate, benzoyl, acetyl, pivaloyl, mesylate, propionyl, valeryl, caproic, caprylic, capric, lauric, myristic, palmitic, stearic, and oleic. Modifications of the active compound, specifically at the N⁶ and 5'-O positions, can affect the bioavailability and rate of metabolism of the active species, thus providing control over the delivery of the active species.

The enantiomerically pure 8-D-dioxolanyl

-7-

purine nucleoside can be converted into a pharmaceutically acceptable ester by reaction with an appropriate esterifying agent, for example, an acid halide or anhydride. The nucleoside or its
5 pharmaceutically acceptable derivative can be converted into a pharmaceutically acceptable salt thereof in a conventional manner, for example, by treatment with an appropriate base. The ester or salt can be converted into the parent nucleoside,
10 for example, by hydrolysis.

The invention as disclosed also includes an asymmetric process for the preparation of enantiomerically pure β -D-dioxolane-nucleosides. The process involves the initial preparation of
15 (2R,4R)- and (2R,4S)-4-acetoxy-2-(protected-oxymethyl)-dioxolane from 1,6-anhydromannose, a sugar that contains all of the necessary stereochemistry for the enantiomerically pure final product, including the correct diastereomeric
20 configuration about the 1 position of the sugar (that becomes the 4'-position in the later formed nucleoside).

The (2R,4R)- and (2R,4S)-4-acetoxy-2-(protected-oxymethyl)-dioxolane is condensed with a
25 desired heterocyclic base in the presence of SnCl_4 , other Lewis acid, or trimethylsilyl triflate in an organic solvent such as dichloroethane, acetonitrile, or methylene chloride, to provide the stereochemically pure dioxolane-nucleoside.

30 Any desired enantiomerically pure β -D-dioxolane purine or pyrimidine nucleoside can be prepared according to the process disclosed herein. The product can be used as a research tool to study the inhibition of HIV in vitro or can be
35 administered in a pharmaceutical composition to inhibit the growth of HIV in vivo.

- 8 -

Brief Description of the Figures

Figure 1A is an illustration of the chemical structure of (\pm)-1-[(2 β ,4 β)-2-(hydroxymethyl)-4-(1,3-thioxolane)]thymine (BCH-189).

5 Figure 1B is an illustration of the chemical structure of (\pm)-1-[(2 β ,4 β)-2-(hydroxymethyl)-4-dioxolanyl]thymine (dioxolane-T).

10 Figure 2 is an illustration of the method of synthesis of enantiomerically pure β -D-(-)-dioxolane-thymine.

15 Figure 3 is an illustration of the method of preparation of a variety of enantiomerically pure β -D-(-)-dioxolanyl purine nucleosides (reagents: (a) TMSOTf, CH₂Cl₂; (b) NH₃, DME; (c) HSCH₂CH₂OH, NaOMe; (d) NH₃, EtOH; (e) n-Bu₄NF, THF.)

Detailed Description of the Invention

As used herein, the term "enantiomerically pure" refers to a nucleoside composition that includes at least 97% of a single enantiomer of that nucleoside.

I. Preparation of Enantiomerically Pure Dioxolane Nucleosides

In preparing enantiomerically pure dioxolane nucleosides, care should be taken to avoid strong acidic conditions that would cleave the dioxolane ring. Reactions should be performed, if possible, in basic or neutral conditions, and when acidic conditions are necessary, the time of reaction should be minimized.

30 A. Preparation of Enantiomerically Pure β -D-Dioxolane-Nucleosides

The key starting material for the synthesis of enantiomerically pure β -D-dioxolane-nucleosides is 1,6-anhydromannose (compound 1, Figure 2). This sugar contains all of the necessary stereochemistry

-9-

position of the sugar (that becomes the 4'-position in the later formed nucleoside). 1,6-Anhydromannose can be prepared according to procedures described in Knauf, A.E.; Hann, R.M.; 5 Hudson, C.S. J. Am. Chem. Soc., 1941, 63, 1447; and Zottola, M.A.; Alonso, R.; Vite, G.D.; Fraser-Reid, B. J. Org. Chem., 1989, 54, 6123. Prior syntheses of dioxolane nucleosides have used racemic mixtures of starting materials for the preparation of the 10 ribose moiety. When the syntheses begin with a racemic mixture of reagents, undesirable racemic mixtures of enantiomeric nucleoside products have been produced. The mixtures are very difficult to separate and significantly increase the cost of the 15 final product. Further, the inclusion of nonnaturally occurring isomers increases the toxicity of the product.

The 1,6-anhydromannose is converted to its isopropylidene derivative with dimethoxyp propane and 20 p-toluenesulfonic acid, which, without isolation, is benzoylated in the 4-position to compound 2 (see Figure 2). An acyl group can also be used to protect the 4-position. The isopropylidene group of compound 2 is then removed by a catalytic amount 25 of an acid such as sulfuric acid, hydrochloric acid, formic acid, trifluoroacetic acid, sulfamic acid, in 60% aqueous dioxane or other suitable organic solvent at a temperature range of approximately 0 to 50°C to give (-)-1,6-anhydro-4- 30 0-benzoyl- β -D-mannopyranose in high yield as a white solid.

In the next step, the glycol of (-)-1,6-anhydro-4-0-benzoyl- β -D-mannopyranose is 35 oxidatively cleaved by treatment with NaIO₄ in H₂O/EtOH (1:1) for one hour at approximately room temperature to produce to the corresponding dialdehyde. Lead tetraacetate can also be used as

-8/1-

for the enantiomerically pure final product (see for example, compound 11, Figure 2), including the correct diastereomeric configuration about the 1

-10-

the oxidizing reagent for this reaction. The dialdehyde is immediately reduced *in situ* with any suitable reducing agent, including NaBH₄, diisobutylaluminum hydride (DIBAL-H), lithium

5 borylhydride (LiBH₄), or sodium bis(2-methoxyethoxy)-aluminum hydride (Red-Al), at approximately room temperature or below. Under the conditions of reaction, compound 4 isomerizes by benzoyl migration from a secondary to a primary position to
10 produce (-)-(2R,4R)-4-(2-benzoxy-1-hydroxyethyl)-2-(hydroxymethyl)-dioxolane (compound 5, Figure 2).

The 2-position of the dioxolane is then protected with a suitable oxygen protecting group, for example, a trisubstituted silyl group such as

15 trimethylsilyl, dimethylhexylsilyl, t-butylidimethylsilyl, t-butyldiphenylsilyl, trityl, alkyl group, acyl groups such as acetyl, propionyl, benzoyl, p-NO₂, benzoyl, or toluyl, methylsulfonyl, or p-toluylsulfonyl. A preferred 20 protecting group is t-butyldiphenylsilyl. After protecting the 2-position of the dioxolane, the benzoyl group is removed from the 2-hydroxyethyl-position with a strong base such as sodium methoxide or ammonia in methanol at approximately 0
25 to 50°C to produce (-)-(2R,4R)-2-(protected-O-methyl)-4-(1,2-dihydroxyethyl)-dioxolane (compound 6, Figure 2) in high yield.

In the next step, the 1,2-dihydroxyethyl group 30 in the 4-position of the dioxolane is converted to a carboxylic acid with an oxidizing agent such as NaIO₄/RuO₂, or lead tetraacetate, at approximately 0 to 50°C to produce (+)-(2R,4R)-2-(protected-oxymethyl)-4-carboxyldioxolane (see compound 7, Figure 2).

35 A modified Hunsdiecker reaction (Dhavale, D.; et al., Tetrahedron Lett., 1988, 29, 6163) is then carried out in ethyl acetate with Pb(OAc)₄ to

-11-

convert (+)-(2R,4R)-2-(protected-oxymethyl)-4-carboxyldioxolane to the corresponding key intermediates (2R,4R)- and (2R,4S)-4-acetoxy-2-(protected-oxymethyl) dioxolane (see compound 8, Figure 2) in good yield.

B. Condensation of a Heterocyclic Base with the Dioxolane Derivative

In the next step of this reaction scheme, the enantiomerically pure dioxolane prepared as described in Section A. is condensed with a protected base in the presence of trimethylsilyl triflate (trimethylsilyl trifluoromethanesulfonate) or a Lewis acid in a dry organic solvent.

Any aromatic compound, and in particular a purine or pyrimidine, containing a nitrogen that is capable of reaction with a center of electron deficiency can be used in the condensation reaction. Purine bases include but are not limited to adenine, hypoxanthine, 2,6-diaminopurine, 6-amino-2-chloropurine, 2-aminopurine, N⁶-alkylpurines, N⁶-benzylpurine, N⁶-halopurine, and guanine. Pyrimidine bases include but are not limited to thymine, cytosine, 6-azapyrimidine, 2-mercaptopurine, and uracil. Functional oxygen and nitrogen groups on the heterocyclic base should be protected before condensation with the sugar if undesired side reactions occur during the synthetic procedure. Protecting groups are well known to those skilled in the art, and include trimethylsilyl, dimethylhexylsilyl, t-butyldimethylsilyl, and t-butyldiphenylsilyl, tritylmethyl, alkyl groups, acyl groups such as acetyl and propionyl, methylsulfonyl, and p-tolylsulfonyl.

Friedel-Crafts catalysts (Lewis acids) that can be used in the condensation reaction include

-12-

SnCl₄, ZnCl₄, TiCl₄, AlCl₃, FeCl₃, BF₃-diethylether, and BCl₃. These catalysts require anhydrous conditions because the presence of water reduces their activity. The catalysts are also inactivated in the presence of organic solvents with active hydrogens, such as alcohols and organic acids. The catalysts are typically used in solvents such as carbon disulfide, methylene chloride, nitromethane, 1,2-dichloroethane, nitrobenzene, tetrachloroethane, chlorobenzene, benzene, toluene, dimethylformamide, tetrahydrofuran, dioxane, or acetonitrile. Anhydrous aluminum chloride is not soluble in carbon disulfide. Niedballa, et al., J. Org. Chem. 39, 25 (1974). The preferred catalyst is SnCl₄. The preferred solvent is 1,2-dichloroethane. Trimethylsilyl triflate can be used under the same conditions described above for the Friedel-Crafts catalysts. The reaction proceeds at a temperature range of from -10°C to 200°C. The choice of catalyst for condensation will affect the final product ratio of α to β nucleoside product. For example, condensation of the intermediates (2R,4R)- and (2R,4S)-4-acetoxy-2-(t-butyldiphenylsilyloxymethyl) dioxolane (compound 8, Figure 2) with silylated thymidine in the presence of trimethylsilyl triflate in CH₂Cl₂ gave a mixture of (-)-1-[(2R,4R)-2-(t-butyldiphenylsilyloxymethyl)-4-dioxolanyl]thymine 9-β (45%) and (+)-1-[(2R,4S)-2-(t-butyldiphenylsilyloxymethyl)-4-dioxolanyl] thymine 10-α (29%). However, the reaction with SnCl₄ produced exclusively β-isomer 9 with trace amounts of α-isomer 10 detectable on TLC.

2,6-Disubstituted purine derivatives were synthesized by the condensation of acetate 8 with the silylated 6-chloro-2-fluoropurine, which gave a mixture (α/β=1/1.3) of 14 and 13 (Figure 3). The

-13-

initially formed N⁷-isomer was again converted to the N⁹-isomer during stirring overnight at room temperature. The analytical sample was obtained from the separation of α,β-mixture to the
5 individual isomers 13 and 14 by a preparative TLC using CH₂CL₂-acetone (19:1) as the developing solvents. However, for the purpose of preparing the final products 21-24, the mixture of 13 and 14 was treated with NH₃ in DME (Robins, M.J.; Vznanski,
10 B. Nucleic acid related compounds. 34. Non-aqueous Diazotization with tert-Butyl nitrite. Introduction of Fluorine, Chlorine, and Bromine at C-2 of Purine Nucleosides. Can. J. Chem. 1981, 2608) to give a mixture of 21-24, which was separated to the individual isomers 15 (24%), 16 (18.6%), 17 (25.8%) and 18 (16%). The guanine 19 and 2,6-diamino 20 derivatives were prepared by the treatment of 15 with 2-mercaptoethanol/NaOMe and ammonia in ethanol, respectively. The free
15 nucleosides 21-26 were obtained upon treatment of the corresponding 5'-silylated nucleosides with n-Bu₄NF in good yields. The α-isomers 23 and 24 were also prepared by the similar procedure as the β-isomers.
20
25 In the final step of this method of preparation of enantiomerically pure (-)-β-D-dioxolane-nucleosides, the 5'-O-position of the nucleoside is deprotected. Desilylation can be carried out with a variety of reagents, including
30 acetic acid, trifluoroacetic acid, hydrogen fluoride, n-tetrabutylammonium fluoride, potassium fluoride and pyridinium HCl. For example, desilylation of compounds 9 and 10 with tetrabutylammonium fluoride gave the desired free
35 nucleosides 11 and 12, respectively (Figure 2). Acetic acid is preferred for commercial scale use because it is inexpensive. Other reagents for

-14-

desilylation are known to those skilled in the art. Deacylation is accomplished in acid or base. 5-O-Ethers can be cleaved with BCl_3 , or trimethylsilyl iodide.

5 The method of preparation of enantiomerically pure β -D-dioxolane-nucleosides is further illustrated in the following working examples. Example 1 sets out in detail a method for the preparation of (2R,4R)- and (2R,4S)-4-acetoxy-2-(*t*-butyldiphenylsilyloxyethyl)dioxolane (compound 8, Figure 2). Example 2 sets out the preparation of (-)-1-[$(2S,4S)$ -2-(hydroxymethyl)-4-dioxolanyl]thymine, referred to as (-)- β -D-dioxolane-T. The enumeration of compounds in
10 Example 2 refer to structures set out in Figure 2. Example 3 provides detailed examples for the preparation of a number of enantiomerically pure β -D-dioxolanyl nucleosides, including (-)-(2R,4R)-2-amino-6-chloro-9-[(2-hydroxymethyl)-1,3-dioxolan-4-yl]purine, (-)-(2R,4R)-9-[(2-hydroxymethyl)-1,3-dioxolan-4-yl]guanine, and (-)-(2R,4R)-2-amino-9-[(2-hydroxymethyl)-1,3-dioxolan-4-yl]adenine.
15
20
25

Example 1 Preparation of (2R,4R)- and (2R,4S)-4-Acetoxy-2-(*t*-butyldiphenylsilyloxyethyl)dioxolane (Compound 8).

(-)-1,6-Anhydro-2,3-isopropylidene-4-O-benzoyl- β -D-mannopyranose

1,6-anhydro- β -D-mannopyranose (compound 1) was mixed with acetone (800 ml) and methanol (300 ml)
30 and stirred for approximately thirty minutes until only a free-flowing solid remained. Dimethoxypropane (300 ml), and p-toluenesulfonic acid (5 g) were then added, and the mixture stirred for 2 hours.

35 The reaction mixture was then made basic with triethylamine (pH 8), and filtered to remove the

-15-

white solid material. The solvents were evaporated, and the residue taken up in ethyl acetate and then crystallized to obtain 4 grams of the 2,3-isopropylidenated product as clear needles.

5 To a solution of the 1,6-anhydro-2,3-isopropylidene- β -D-mannopyranose (5.01 g, 0.025 mol) in pyridine (40 ml) was added dropwise benzoyl chloride (3.74 ml, 0.062 mol) at 0°C. The mixture was stirred for 45 minutes at 0°C. Ice was then
10 added to the reaction mixture to remove excess benzoyl chloride. The solvent was evaporated under vacuum and the residue was dissolved in ethyl acetate (200 ml). The organic layer was washed with water, sat. NaHCO₃, and brine. The resulting
15 material was dried over anhydrous MgSO₄, filtered, and then evaporated to give (-)-1,6-anhydro-2,3-isopropylidene-4-O-benzoyl- β -D-mannopyranose crude product (compound 2, 8.7 g) as yellowish solid.

(-)-1,6-Anhydro-4-O-benzoyl- β -D-mannopyranose (3).

20 To a solution of 1,6-anhydro-4-O-benzoyl-2,3-isopropylidene- β -D-mannopyranose 2 (10.0 g, 32.6 mmole) in 60% aqueous dioxane (820 ml) was added concentrated H₂SO₄ (3.36 ml). The mixture was stirred at 70-80° C for 15 hours, and then cooled
25 in an ice bath, neutralized with NaHCO₃, and concentrated until half of the original volume remained. The solution was then extracted with ethyl acetate and the combined organic layers washed with saturated NaHCO₃, solution and water, dried, and evaporated to give 3 as a white solid.
30 The solid was crystallized from CH₂Cl₂-n-hexane to yield 3 (7.4 g, 85.3%) as white solid: [α]²⁵D-154.7° (C, 0.21 MeOH); ¹H NMR (DMSO-d₆): δ 3.56-4.61 (m, 5H, 2,3,5,6-H), 4.82 (d, J=8.1 Hz, 1H, OH D₂O exchangeable), 5.02 (s, 1H, 4-H), 5.09 (d, J=3.7

-16-

Hz, 1H, OH, D₂O exchangeable), 5.28 (s, 1H, 1-H), 7.46-8.05 (m, 5H, Ar-H); IR (KBr) 3410, 1710 cm⁻¹; Anal. Calcd for C₁₃H₁₄O₆: C, 58.64; H, 5.31. Found: C, 58.51; H, 5.34.

5 (-)-(2R,4R)-4-(2-Benzoyl-1-hydroxyethyl)-2-(hydroxymethyl)dioxolane (5).

To a solution of 3 (7.4 g, 27.8 mmole) in 95% ethanol (200 ml) was added a solution of NaIO₄ (6.54 g, 30.7 mmole) in water (200 ml). The mixture was 10 stirred at room temperature for 1 hour. After checking to insure the complete conversion of diol to dialdehyde by thin layer chromatography, the reaction mixture was concentrated to the half of the original volume. Methanol (200 ml) was added 15 to the residue and the mixture was cooled to 50°C. Sodium borohydride (4.2 g, 111.0 mmole) was added to the mixture portion-wise for 5 minutes and the mixture was stirred at 50°C for 10 minutes, neutralized with glacial acetic acid and 20 concentrated to yield crude 3 as yellow oil. The oil was purified by column chromatography over silica gel to yield pure 3 as colorless oil, that was crystallized from diethyl ether/n-hexane to yield 5 (6.12 g, 82%) as white solid: [α]²⁵D - 18.5° 25 (C 0.20, methanol); ¹H NMR (DMSO-d₆): δ 3.47 (dd, J=5.9, 3.7 Hz, 2H, CH₂OH), 3.72-4.14 (m, 4H, 4, 5-H and CHOH), 4.27-4.95 (m, 2H, CH₂OBz), 4.81-4.95 (m, 2-H and pri OH), 5.43 (d, J=5.5 Hz, 1H, sec OH, D₂O exchangeable), 7.43-8.09 (m, 5H, Ar-H); Anal. Calcd for C₁₃H₁₆O₆: C, 58.19; H, 6.02. Found: C, 58.09; H, 6.01.

-17-

(-)-(2R,4R)-4-(2-Benzoxo-1-hydroxyethyl)-2-(t-butyldiphenylsilyloxy-methyl)-dioxolane.

To a solution of 3 (2.8 g, 10.4 mmole) and imidazole (2.04 g, 30.0 mmole) in dimethylformamide (40 ml) was added t-butyldiphenylsilyl chloride (3 ml, 11.5 mmole). The mixture was stirred at room temperature for 2 hours. The reaction mixture was evaporated to yield a yellow oil, that was purified by column chromatography over silica gel to yield 4 (4.48 g, 85%) as a colorless oil; $[\alpha]^{25}\text{D} = 14.2^\circ$ (C 0.26, methanol); ^1H NMR (DMSO-d₆): δ 1.00 (s, 9H, t-Bu), 3.68-3.87 (m, 3H, CH₂OTBDPS and CHOH), 3.98-4.16 (m, 3H, 4,5-H), 4.20-4.55 (m, 2H, CH₂OBz), 5.07 (t, J=3.3 Hz, 1H, 2-H), 5.47 (d, J=5.7 Hz, 1H, OH, D₂O exchangeable), 7.40-8.33 (m, 10H, Ar-H); Anal. Calcd for C₂₉H₃₄O₆Si: C, 68.73; H, 6.79. Found: C, 68.86; H, 6.83.

(-)-(2R,4R)-2-(t-Butyldiphenylsilyloxyethyl)-4-(1,2-dihydroxyethyl)-dioxolane (6).

To a solution of (-)-(2R,4R)-4-(2-benzoxo-1-hydroxyethyl)-2-(t-butyldiphenylsilyloxy-methyl)-dioxolane (2.52 g, 5.0 mmole) in methanol (40 ml) was added a 0.078 M solution of sodium methoxide (7.3 ml) in methanol. The mixture stirred at room temperature for two hours. The mixture was neutralized with acetic acid and concentrated. The residue was then portioned between ethyl acetate and water, and the aqueous layer extracted with ethyl acetate. The combined organic layers were washed with a saturated NaHCO₃ solution and then water, and then dried, evaporated, and purified by column chromatography over silica gel to yield 6 (1.9 g, 95%) as colorless oil: $[\alpha]^{25}\text{D}=2^\circ$ (C 0.25, MeOH); ^1H NMR (DMSO-d₆) δ 1.00 (s, 9H, t-Bu), 3.40-3.52 (m, 3H, CH₂OH and CHOH), 3.64 (d, J=3.7 Hz, 2H,

-18-

CH₃OTBDPS), 3.82-3.95 (m, 3H, 4.5-H), 4.49 (t, J=5.3 Hz, 1H, pri OH, D₂O exchangeable), 4.82 (d, J=5.1 Hz, 1H, sec OH, D₂O exchangeable), 5.01 (t, J=3.7 Hz, 1H, 2-H), 7.36-7.71 (m, 10H, Ar-H); Anal. Calcd 5 for C₂₂H₃₃H₃₀O₅Si: C, 65.63; H, 7.53. Found: C, 65.72; H, 7.52.

(+)-(2R,4R)-2-(t-Butyldiphenylsilyloxyethyl)-4-carboxyldioxolane (7).

To a biphasic solution of 6 (1.6 g, 4.0 mmole) 10 in CH₃CN (8 ml), CCl₄ (8 ml) and H₂O (12 ml) was added NaIO₄ (3.59 g, 16.8 mmole) and RuO₂ hydrate (8.5 mg). The mixture was vigorously stirred at room temperature for 5 hours. Methylene chloride (40 ml) was added to the mixture. The organic 15 layer was separated. The aqueous layer was extracted with CH₂Cl₂. The combined organic layers were washed with water, filtered through celite pad and then concentrated to yield crude 7 (1.2 g, 77.4%) as black oil, that was used in the next reaction without further purification. For 20 analytical purposes crude 7 was purified by column chromatography over silica gel to yield 7 as a white foam: [α]²⁵D + 15.7° (C 0.28, MeOH); ¹H NMR (DMSO-d₆) δ 0.99 (s, 9H, t-Bu), 3.43-4.05 (m, 4H, 5-H and CH₃OTBDPS), 4.25 (t, J=6.8 Hz, 1H, 4-H), 5.04 25 (dd, J=5.1, 3.7 Hz, 1H, 2-H), 7.38-7.72 (m, 10H, Ar-H).

(2R,4R)- and (2R,4S)-4-Acetoxy-2-(t-butyldiphenylsilyloxyethyl) dioxolane (8).

To a solution of 7 (0.46 g, 1.14 mmole) in ethyl acetate (10 ml) was added pyridine (0.09 ml, 1.25 mmole) and Pb(OAc)₄ (0.66 g, 1.49 mmole). The mixture was stirred at room temperature for 15 hours under N₂, and then filtered through celite

-19-

pad, and then concentrated and purified by column chromatography over silica gel to yield 8 (0.29 g, 63.5%) as a colorless oil: ^1H NMR (CDCl_3) δ 1.06 and 1.10 (s, 9H, t-Bu), 1.92 and 2.06 (s, 1H, CH_3), 5 3.71-4.24 (m, 4H, 5-H and CH_2OTBDPS), 5.25 and 5.38 (t, $J=4.3$ and 3.3 Hz each, 1H, 2-H), 6.27-6.41 (m, 1H, 4-H), 7.20-7.72 (m, 10H, Ar-H); IR (KBr) 3400, 1620 cm^{-1} .

10 Example 2 Preparation of (-)-1-[(2R,4R)-2-(Hydroxymethyl)-4-dioxolanyl]thymine (11).

15 (-)-1-[(2R,4R)-2-(t-Butyldiphenylsilyloxyethyl)-4-dioxolanyl]thymine (9) and (+)-1-[(2R,4S)-2-(t-Butyldiphenylsilyloxyethyl)-4-dioxolanyl] thymine (10).

To a suspension of thymine (0.15 g, 1.2 mmole) in hexamethyldisilazane (10 ml) was added a catalytic amount of $(\text{NH}_4)_2\text{SO}_4$, and the mixture refluxed for 3 hours. The clear solution obtained 20 was concentrated to yield silylated thymine as a colorless oil. A solution of 8 (0.24 g, 0.6 mmole) in CH_2Cl_2 (5 ml) was added to a solution of silylated thymine in CH_2Cl_2 (5 ml) and the mixture cooled to 5°C. To the cooled mixture was added 25 trimethylsilyl triflate (0.23 ml, 1.2 mmole), and the mixture stirred at room temperature for 1 hour under N_2 . A saturated NaHCO_3 solution (20 ml) was added to the mixture, and the mixture again stirred at room temperature for 30 minutes. The organic 30 layer was then separated and the aqueous layer extracted with CH_2Cl_2 . The combined organic layer was washed with a saturated NaHCO_3 solution and water, dried, concentrated and separated by column chromatography over silical gel to yield 9 (0.125 g, 44.6%) as white foam and 10 (0.08 g, 28.6%) as 35 white foam: 9 (β -form); $[\alpha]^{25}\text{D} - 6.98^\circ$ (C 0.43,

-20-

MeOH); ^1H NMR (CDCl_3) δ 1.08 (s, 9H, t-Bu), 1.67 (s, 3H, CH_3), 3.92 (d, $J=3.2$ Hz, 2H, CH_2OTBDPS), 4.14 (d, $J=4.0$ Hz, 2H, 5-H), 5.06 (t, $J=3.2$ Hz, 1H, 2-H), 6.36 (t, $J+4.0$ Hz, 1H, 4-H), 7.26-7.75 (m, 10H, 5 Ar-H), 9.51 (br s, 1H, H=NH): UV (MeOH) λ_{max} 265.0 (pH 2); 264.4 nm (pH 11); Anal. Calcd for $\text{C}_{25}\text{H}_{30}\text{O}_3\text{N}_2\text{Si}$: C, 64.34; H, 6.49; N, 6.00. Found C, 64.28; H, 6.51; N, 5.98:

10 (α -form); $[\alpha]^{25}\text{D} + 11.3^\circ$ (C 0.23, MeOH); ^1H NMR (CDCl_3) δ 1.08 (s, 9H, t-Bu), 1.94 (d, $J=1.2$ Hz, 3H, CH_3), 3.70 (d, $J=3.2$ Hz, 2H, CH_2OTBDPS), 4.01 (dd, $J=9.5$, 2.3 Hz, 1H, 5H), 4.35 (dd, $J=9.5$, 5.3 Hz, 1H, 5-H), 5.55 (t, $J=3.2$ Hz, 1H, 2-H), 6.32 (dd, $J=5.3$, 2.3 Hz, 1H, 4-H), 7.17 (d, $J=1.2$ Hz, 1H, 6'-H), 7.37-7.74 (m, 10H, Ar-H), 9.57 (br s, 1H, NH); UV (MeOH) λ_{max} 265.0; (pH 2); 264.5 nm (pH 11); Anal. Calcd for $\text{C}_{25}\text{H}_{30}\text{O}_3\text{N}_2\text{Si}$: C, 64.34; H, 6.49; N, 6.00. Found C, 64.23; H, 6.51; N, 5.93.

20 (-)-1-[(2R,4R)-2-(Hydroxymethyl)-4-dioxolanyl]thymine (11).

To a solution of 9 (93.3 mg, 0.2 mmole) in tetrahydrofuran (THF) (3 ml) was added a 1.0 M solution of tetra-n-butylammonium fluoride in THF (0.24 ml, 0.24 mmole) and the mixture stirred at room temperature for 1 hour. The mixture was then concentrated and purified by column chromatography over silica gel to yield 11 (42 mg, 92.1%) as white solid: $[\alpha]^{25}\text{D}-18.8^\circ$ (C 0.17, MeOH); ^1H NMR (DMSO-d_6) δ 1.75 (d, $J=1.2$ Hz, 3H, CH_3), 3.63 (dd, $J+6.0$, 2.6 Hz, 2H, CH_2OH), 4.03 (dd, $J=9.9$, 5.5 Hz, 1H, 5-H), 4.22 (dd, $J=9.9$, 2.0 Hz, 1H, 5-H), 4.90 (t, $J=2.6$ Hz, 1H, 2-H), 5.16 (t, $J=t.0$ Hz, 1H, OH), 6.21 (dd, $J=5.5$, 2.0 Hz, 1H, 4-H), 7.67 (d, $J=1.2$ Hz, 1H, 6'-H), 11.27 (br s, 1H NH); UV (H₂) λ_{max} 266.0 (ϵ 10757); 266.5 (ϵ 9894) (pH 2); 266.3 (ϵ 8397) (pH 11); Anal. Calcd for $\text{C}_9\text{H}_{12}\text{O}_3\text{N}_2$: C, 47.36; H, 5.31; N, 11).

-21-

12.28. found: C, 47.28; H, 5.34; N, 12.29.

(+)-1-[(2R,4S)-2-(Hydroxymethyl)-4-dioxolanyl]thymine (12).

5 Deprotection of 10 (60 mg, 0.13 mmole) according to same procedure as described above for 11 yielded 12 (26 mg, 87.6%) as a white foam: $[\alpha]^{25}\text{D} + 10.7^\circ$ (C 0.15, MeOH); ^1H NMR (DMSO-d₆) δ 1.79 (s, 3H, CH₃), 3.43 (dd, J=6.0, 3.7 Hz, 2H, CH₂OH), 4.02 (dd, J=9.5, 3.3 Hz, 1H, 5-H), 4.28 (dd, J=9.5, 5.6 Hz, 10 1H, 5-H), 5.00 (t, J=6.0 Hz, 1H, OH), 5.47 (t, J=3.7 Hz, 1H, 2-H), 6.17 (dd, J=5.6, 3.3 Hz, 1H, 4-H), 7.43 (d, J=1.2 Hz, 1H, 6'-H), 11.32 (br s, 1H NH); UV (H₂O) λ_{max} 266.5 (ϵ 9454); 266.5 (ϵ 9199) (pH 2); 266.3 (ϵ 6925) (pH=11); Anal. Calcd for C₉H₁₂O₅N₂; 15 C, 47.36; H, 5.31; N, 12.28. found: C, 47.22; H, 5.32; N, 12.16.

Example 3 Preparation of Enantiomerically Pure S-D-Dioxolanyl Purine Nucleosides

20 (2R,4R) and (2R,4S)-9-[[2-[(tert-Butyldiphenylsilyl)oxy]methyl]-1,3-dioxolan-4-yl]-6-chloro-2-fluoropurine (13 and 14).

A mixture of 2-fluoro-6-chloropurine (4.05 g, 23.47 mmol) and ammonium sulfate (catalytic amount) in hexamethyldisilazane (940 mL) was refluxed for 25 hours. The resulting solution was concentrated under anhydrous conditions to yield silylated 2-fluoro-6-chloropurine as a white solid. To a cooled (0°C) and stirred solution of silylated 2-fluoro-6-chloropurine (5.69 g, 23.69 mmol)b and compound 8 (7.84 g, 19.57 mmol) in dry methylene chloride (175 mL) was added TMSOTf (4.41 mL, 23.44 mmol). The reaction mixture was warmed to room temperature and stirred for 16 hours, during which time, all the initially formed N, condensed product

-22-

was converted to N₉-isomer. The reaction mixture was quenched with saturated NaHCO₃ solution (50 mL) and stirred for an additional 20 minutes at room temperature, evaporated to dryness under reduced pressure. The residue was dissolved in ethyl acetate (200 mL), washed with water and brine, dried (anhydrous Na₂SO₄), filtered and evaporated to give a solid residue, which was purified by silica gel column chromatography (20% EtOAc in hexanes) to afford a mixture of β-anomer 19 and α-anomer 20 (1.3:1; β/α) as a white crystalline solid (6.30 g, 62.8%). The analytical sample was purified by preparative TLC using CH₂Cl₂-acetone (19:1) as the developing system to give 13 (R_f = 0.5p0) and 14 (R_f = 0.55) for NMR characterization: UV (MeOH) λ_{max} 269.0 nm.

(-)-(2R,4R)-2-Amino-9-[(2-[(tert-butylidiphenylsilyl)oxy]methyl]-1,3-dioxolan-4-yl]-6-chloropurine (15), (-)-(2R,4R)-9-[(2-[(tert-butylidiphenylsilyl)oxy]methyl]-1,3-dioxolan-4-yl]-2-fluoroadenine (16), (+)-(2R,4S)-2-Amino-9-[(2-[(tert-butylidiphenylsilyl)oxy]methyl]-1,3-dioxolan-4-yl]-6-chloropurine (17) and (+)-(2R,4S)-9-[(2-[(tert-butylidiphenylsilyl)oxy]methyl]-1,3-dioxolan-4-yl]-2-fluoroadenine (18).

Dry ammonia gas was bubbled into a stirred solution of 13 and 14 (6.25 g, 12.18 mmol) in DME (125 mL) overnight). The solvent was evaporated under reduced pressure and the residue was subjected to chromatographic separation of the four compounds on a silica gel column (20-30% ethyl acetate in CH₂Cl₂). 15 (R_f = 0.35, 1.49 g, 24%): a white crystalline solid. UV (MeOH) λ_{max} 309.5 nm. Anal. (C₂₅H₂₈CIN₅O₃Si) C, H, Cl, N. 16 (R_f = 0.21, 1.12 g, 18.6%): colorless needles. UV (MeOH) λ_{max} 261.0, 268.0 (sh) nm. Anal. (C₂₅H₂₈FN₅O₃Si) C, H, F, N. 17 (R_f = 0.43, 1.60 g, 25.76%): a white crystalline solid. UV (MeOH) λ_{max} 261.0, 269.0 (sh) nm. Anal.

-24-

desilylation according to the same procedure described for 23 to give compound 25 (0.07 g, 61%) as a microcrystalline solid, which was recrystallized from MeOH: UV (H₂O) λ_{max} 252.0

5 (ε_{8,730}) (pH 7), 254.4 (ε_{12,130}), 277.5 (sh) (ε_{8,070}) (pH 2), 264.3 (ε_{10,800}) (pH 11). Anal. (C₉H₁₁N₅O₄) C, H, N.

(--)(2R,4R)-2-Amino-9-[(2-hydroxymethyl)-1,3-dioxolan-4-yl]adenine (26).

10 A steel bomb was charged with compound 15 (0.28 g, 0.55 mmol), anhydrous ethanol (20 mL) saturated with NH₃, and heated at 90°C for 6 hours. After cooling, the compound 20 (0.26 g, 95%) obtained on evaporation of the solvent in vacuo, and then
15 desilylated according to the same procedure described for preparation of 23 to give 26 (0.10 g, 75%) as white micro needles, recrystallized from MeOH: UV (H₂O) λ_{max} 279.0 nm (ε 8,040) (pH 7), 290.0 (ε 7,070) (pH 2), 278.8 (ε 7,580) (pH 11). Anal.
20 (C₉H₁₂N₆O₃) C, H, N.

(--)(2R,4R)-2-Amino-9-[(2-hydroxymethyl)-1,3-dioxolan-4-yl]purine can be prepared by reduction of compound 21 using a variety of reducing agents, including palladium on carbon and hydrogen gas or 25 tributyltin hydride and azabisisobutyronitrile.

II. Anti-HIV Activity of Dioxolane Nucleosides

B-D-Dioxolane-nucleosides can be used as research tools to inhibit the growth of HIV in vitro, or can be administered to humans 30 pharmaceutically to inhibit the growth of HIV in vivo.

The ability of B-D-dioxolane-nucleosides to inhibit HIV can be measured by various experimental techniques. The technique used herein, and 35 described in detail below, measures the inhibition of viral replication in phytohemagglutinin (PHA)

-25-

stimulated human peripheral blood mononuclear (PBM) cells infected with HIV-1 (strain LAV). The amount of virus produced is determined by measuring the virus-coded reverse transcriptase enzyme. The
5 amount of enzyme produced is compared to an HIV control. The method is described in detail below.

Antiviral and Cytotoxic Assay in Human Peripheral Blood Mononuclear Cells.

A. Three-day-old phytohemagglutinin-stimulated
10 PBM cells (10^6 cells/ml) from hepatitis B virus and HIV-1 seronegative healthy donors were infected with HIV-1 (strain LAV) at a concentration of about 100 times the 50% tissue culture infectious dose (TCID₅₀) per ml and cultured in the presence and
15 absence of various concentrations of antiviral compounds.

B. Approximately 45 minutes after infection, the medium, with the compound to be tested (2 times the final concentration in medium) or without compound, was added to the flasks (5ml; final volume 10 ml). AZT was used as a positive control.
20

C. The cells were exposed to the virus (about 2×10^3 dpm/ml, as determined by reverse transcriptase assay) and then placed in a CO₂ incubator. HIV-1 (strain LAV) was obtained from the Center for Disease Control, Atlanta, Georgia. The methods used for culturing the PBM cells, harvesting the virus and determining the reverse transcriptase activity were those described by McDougal et al.
25 (J. Immun. Meth. 76, 171-183, 1985) and Spira et al. (J. Clin. Meth. 25, 97-99, 1987), except that fungizone was not included in the medium (see Schinazi, et al., Antimicrob. Agents Chemother. 32, 1784-1787 (1988)). The reverse transcriptase
30 activity in the virus-infected control was about 2×10^3 dpm per ml. Blank and uninfected cell control
35

-26-

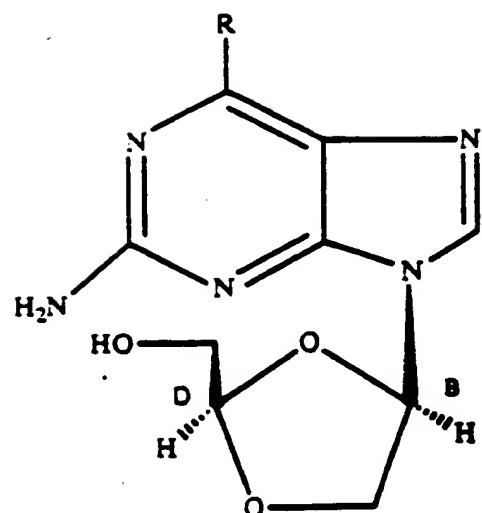
values were about 300 and 1,000 dpm, respectively. Similar results are obtained when Step C is performed before step B.

D. On day 6, the cells and supernatant were
5 transferred to a 15 ml tube and centrifuged at
about 900 g for 10 minutes. Five ml of supernatant
were removed and the virus was concentrated by
centrifugation at 40,000 rpm for 30 minutes
(Beckman 70.1 Ti rotor). The solubilized virus
10 pellet was processed for determination of the
levels of reverse transcriptase. Results are
expressed in dpm/ml of sampled supernatant.

The percent inhibition of virus, as determined
from measurements of reverse transcriptase, is
15 plotted versus the micromolar concentration of
compound. The EC₅₀ is the concentration of compound
at which there is a 50% inhibition of viral growth.

Using this assay, it has been discovered that a
small number of 8-D-dioxolanyl purine nucleosides
20 are potent anti-HIV agents. Specifically, as
indicated in Table 1, compounds 21, 25, and 26
exhibit a low effective median concentration,
ranging from 0.027 to 0.69 μ M.

-27-

Table 1

R	Anomer	EC_{50}^*
Cl	B-D	0.9
Cl	B-L	13.4
NH_2	B-D	0.7
OH	B-D	0.03

* Mean of at least 2 assays, using different donor cells.
Standard error estimated at plus or minus 10%.

-28-

In contrast to the previous report that β -D-(\pm)-dioxolane-thymine has low efficacy against HIV in ATH8 cells, the enantiomerically pure β form 11 exhibited a potent anti-HIV activity ($EC_{50} = 0.3$ μM). It was surprising to discover that enantiomerically pure β -D-(-)-dioxolane-T has significantly higher anti-HIV activity than the racemic mixture of the compound. This difference may be explained based on the rate of phosphorylation of 11 in these systems. As expected, the α -isomer 12 did not exhibit any significant anti-HIV activity. The EC_{50} of (-)-1-[$(2S,4S)$ -2-(hydroxymethyl)-4-dioxolan-1-yl]thymine in PBM cells was measured as 0.2 μM .

15 III. Toxicity of Dioxolane Nucleosides

The toxicities of compounds 21, 25, and 26, were evaluated in uninfected human PBM cells, CEM cells (T-lymphoblastoid cell line obtained from ATCC, Rockville, MD) and Vero (African Green Monkey kidney) cells. The three compounds were not toxic in any of the cell lines at a concentration of 100 μM .

IV. Preparation of Pharmaceutical Compositions

Humans suffering from HIV infection can be treated by administering to the patient an effective amount of (-)-(2R,4R)-2-amino-6-chloro-9-[(2-hydroxymethyl) -1,3-dioxolan-4-yl]purine; (-)-(2R,4R)-9-[(2-hydroxymethyl) -1,3-dioxolan-4-yl]guanine; (-)-(2R,4R)-2-amino-9-[(2-hydroxymethyl) -1,3-dioxolan-4-yl]adenine; or (-)-(2R,4R)-2-amino-9-[(2-hydroxymethyl) -1,3-dioxolan-4-yl]purine or a pharmaceutically acceptable derivative or salt thereof, optionally in a

-29-

pharmaceutically acceptable carrier or diluent. The active materials can be administered by any appropriate route, for example, orally, parenterally, intravenously, intradermally, 5 subcutaneously, or topically, in liquid or solid form.

The active compound is included in the pharmaceutically acceptable carrier or diluent in an amount sufficient to deliver to a patient a 10 therapeutically effective amount without causing serious toxic effects in the patient treated.

A preferred dose of the active compound for all of the above-mentioned conditions will be in the range from about 1 to 60 mg/kg, preferably 1 to 20 15 mg/kg, of body weight per day, more generally 0.1 to about 100 mg per kilogram body weight of the recipient per day. The effective dosage range of the pharmaceutically acceptable derivatives can be calculated based on the weight of the parent 20 nucleoside to be delivered. If the derivative exhibits activity in itself, the effective dosage can be estimated as above using the weight of the derivative, or by other means known to those skilled in the art.

25 The compound is conveniently administered in unit any suitable dosage form, including but not limited to one containing 7 to 3000 mg, preferably 70 to 1400 mg of active ingredient per unit dosage form. A oral dosage of 50-1000 mg is usually 30 convenient.

Ideally the active ingredient should be 35 administered to achieve peak plasma concentrations of the active compound of from about 0.2 to 70 μ M, preferably about 1.0 to 10 μ M. This may be achieved, for example, by the intravenous injection of a 0.1 to 5% solution of the active ingredient, optionally in saline, or administered as a bolus of

-30-

the active ingredient.

The concentration of active compound in the drug composition will depend on absorption,

inactivation, and excretion rates of the drug as

5 well as other factors known to those of skill in the art. It is to be noted that dosage values will also vary with the severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage

10 regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that the concentration ranges set forth herein are exemplary

15 only and are not intended to limit the scope or practice of the claimed composition. The active ingredient may be administered at once, or may be divided into a number of smaller doses to be administered at varying intervals of time.

20 A preferred mode of administration of the active compound is oral. Oral compositions will generally include an inert diluent or an edible carrier.

They may be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral

25 therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules.

Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition.

30 The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or

35 gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as

-31-

magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange 5 flavoring. When the dosage unit form is a capsule, it can contain, in addition to material of the above type, a liquid carrier such as a fatty oil. In addition, dosage unit forms can contain various other materials which modify the physical form of 10 the dosage unit, for example, coatings of sugar, shellac, or other enteric agents.

The active compound or pharmaceutically acceptable salt or derivative thereof can be administered as a component of an elixir, 15 suspension, syrup, wafer, chewing gum or the like. A syrup may contain, in addition to the active compounds, sucrose as a sweetening agent and certain preservatives, dyes and colorings and flavors.

20 The active compound, or pharmaceutically acceptable derivative or salt thereof can also be mixed with other active materials that do not impair the desired action, or with materials that supplement the desired action, such as antibiotics, 25 antifungals, antiinflammatories, or other antivirals, including other nucleoside anti-HIV compounds.

Solutions or suspensions used for parenteral, intradermal, subcutaneous, or topical application 30 can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl 35 alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers

-32-

such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. The parental preparation can be enclosed in ampoules, disposable syringes or
5 multiple dose vials made of glass or plastic.

If administered intravenously, preferred carriers are physiological saline or phosphate buffered saline (PBS).

In a preferred embodiment, the active compounds
10 are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used,
15 such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained
20 commercially from Alza Corporation and Nova Pharmaceuticals, Inc.

Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) are also preferred as pharmaceutically acceptable carriers. These may be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811 (which is incorporated herein by reference in its entirety). For example,
25 liposome formulations may be prepared by dissolving appropriate lipid(s) (such as stearoyl phosphatidyl ethanolamine, stearoyl phosphatidyl choline, arachadoyl phosphatidyl choline, and cholesterol) in an inorganic solvent that is then evaporated,
30 leaving behind a thin film of dried lipid on the surface of the container. An aqueous solution of
35 the active compound or its monophosphate,

-33-

diphosphate, and/or triphosphate derivatives are then introduced into the container. The container is then swirled by hand to free lipid material from the sides of the container and to disperse lipid aggregates, thereby forming the liposomal suspension.

V. Preparation of Phosphate Derivatives of 8-D-Dioxolane-Nucleosides

Mono, di, and triphosphate derivative of 8-D-dioxolane-nucleosides can be prepared as described below.

The monophosphate can be prepared according to the procedure of Imai et al., J. Org. Chem., 34(6), 1547-1550 (June 1969). For example, about 100 mg of 8-D-dioxolane-nucleoside and about 280 μ l of phosphoryl chloride are reacted with stirring in about 8 ml of dry ethyl acetate at about 0°C for about four hours. The reaction is quenched with ice. The aqueous phase is purified on an activated charcoal column, eluting with 5% ammonium hydroxide in a 1:1 mixture of ethanol and water. Evaporation of the eluant gives ammonium-(8-D-dioxolane-nucleoside)-5'-monophosphate.

The diphosphate can be prepared according to the procedure of Davisson et al., J. Org. Chem., 52(9), 1794-1801 (1987). 8-D-Dioxolane-nucleosides can be prepared from the corresponding tosylate, that can be prepared, for example, by reacting the nucleoside with tosyl chloride in pyridine at room temperature for about 24 hours, working up the product in the usual manner (e.g., by washing, drying, and crystallizing it).

The triphosphate can be prepared according to the procedure of Hoard et al., J. Am. Chem. Soc., 87(8), 1785-1788 (1965). For example, 8-D-dioxolane-nucleoside is activated (by making a imidazolide, according to methods known to those

-34-

skilled in the art) and treating with tributyl ammonium pyrophosphate in DMF. The reaction gives primarily the triphosphate of the nucleoside, with some unreacted monophosphate and some diphosphate.

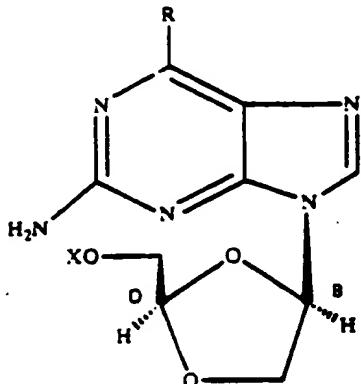
5 Purification by anion exchange chromatography of a DEAE column is followed by isolation of the triphosphate, e.g., as the tetrasodium salt.

This invention has been described with reference to its preferred embodiments. Variations and 10 modifications of the invention, enantiomerically pure 8-D-dioxolane-nucleosides, will be obvious to those skilled in the art from the foregoing detailed description of the invention. It is intended that all of these variations and 15 modifications be included within the scope of the appended claims.

-35-

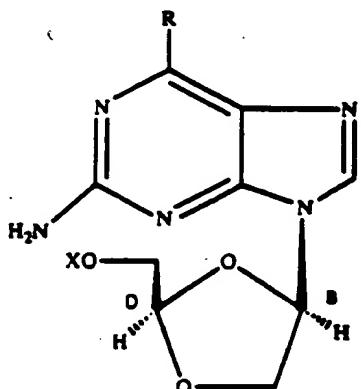
WE CLAIM:

1. A method for the treatment of HIV infection in humans, comprising administering an HIV treatment amount of an enantiomerically pure β -D-dioxolanyl nucleoside of the structure:



wherein R is OH, and X is selected from the group consisting of hydrogen, alkyl, acyl, monophosphate, diphosphate, and triphosphate, or its pharmaceutically acceptable salt, and wherein the compound is 97% free of the corresponding β -L enantiomer.

2. A method for the treatment of HIV infection in humans, comprising administering an HIV treatment amount of an enantiomerically pure β -D-dioxolanyl nucleoside of the structure:

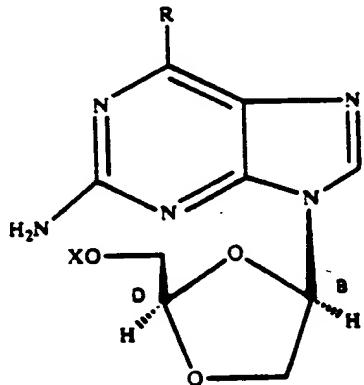


wherein R is NH₂, and X is selected from the group consisting of hydrogen, alkyl, acyl, monophosphate, diphosphate, and triphosphate, or its

-36-

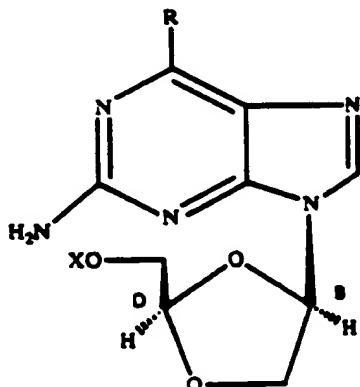
pharmaceutically acceptable salt, and wherein the compound is 97% free of the corresponding *S*-L enantiomer.

3. A method for the treatment of HIV infection in humans, comprising administering an HIV treatment amount of an enantiomerically pure *S*-D-dioxolanyl nucleoside of the structure:



wherein R is H or Cl, and X is selected from the group consisting of hydrogen alkyl, acyl, monophosphate, diphosphate, and triphosphate, or its pharmaceutically acceptable salt, and wherein the compound is 97% free of the corresponding *S*-L enantiomer.

4. A pharmaceutical composition comprising an effective amount of an enantiomerically pure *S*-D-dioxolanyl nucleoside of the structure:

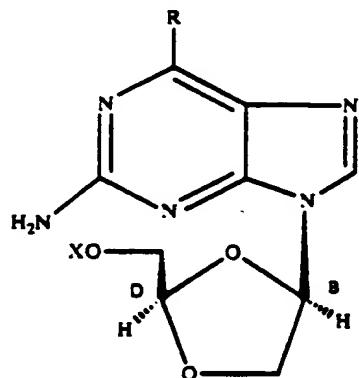


wherein R is OH, and X is selected from the group consisting of hydrogen, alkyl, acyl, monophosphate, diphosphate, and triphosphate, or its

-37-

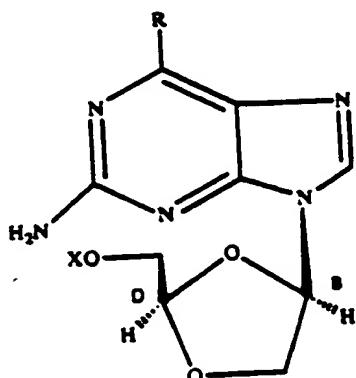
pharmaceutically acceptable salt, and wherein the compound is 97% free of the corresponding *S*-L enantiomer, in a pharmaceutically acceptable carrier or diluent.

5. A pharmaceutical composition comprising an effective amount of an enantiomerically pure *S*-D-dioxolanyl nucleoside of the structure:



wherein R is NH₂, and X is selected from the group consisting of hydrogen, alkyl, acyl, monophosphate, diphosphate, and triphosphate, or its pharmaceutically acceptable salt, and wherein the compound is 97% free of the corresponding *S*-L enantiomer, in a pharmaceutically acceptable carrier or diluent.

6. A pharmaceutical composition comprising an effective amount of an enantiomerically pure *S*-D-dioxolanyl nucleoside of the structure:

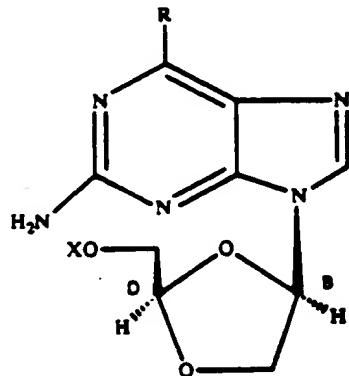


wherein R is H or Cl, and X is selected from the group consisting of hydrogen, alkyl, acyl,

-38-

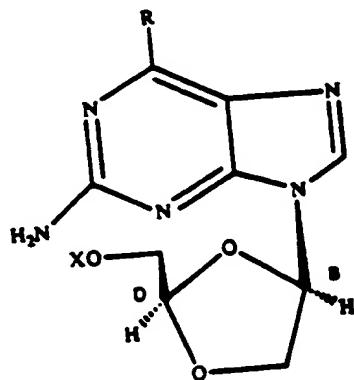
monophosphate, diphosphate, and triphosphate, or its pharmaceutically acceptable salt, and wherein the compound is 97% free of the corresponding S-L enantiomer.

7. An enantiomerically pure S-D-dioxolanyl nucleoside of the structure:



wherein R is OH, and X is selected from the group consisting of hydrogen, alkyl, acyl, monophosphate, diphosphate, and triphosphate, and wherein the compound is 97% free of the corresponding S-L enantiomer, in a pharmaceutically acceptable carrier or diluent.

8. An enantiomerically pure S-D-dioxolanyl nucleoside of the structure:

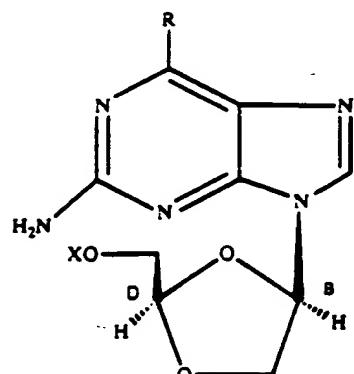


wherein R is NH₂, and X is selected from the group consisting of hydrogen, alkyl, acyl, monophosphate, diphosphate, and triphosphate, and wherein the compound is 97% free of the corresponding S-L enantiomer, in a pharmaceutically acceptable

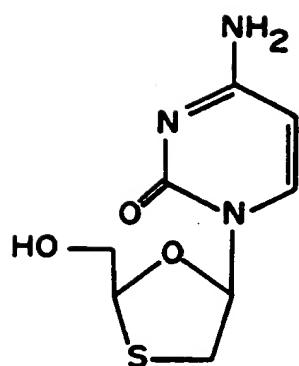
-39-

carrier or diluent.

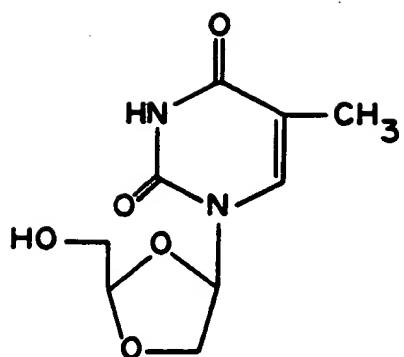
9. An enantiomerically pure β -D-dioxolanyl nucleoside of the structure:



wherein R is H or Cl, and X is selected from the group consisting of hydrogen, alkyl, acyl, monophosphate, diphosphate, and triphosphate, and wherein the compound is 97% free of the corresponding β -L enantiomer.



(±)-BCH - 189



(±)-Dioxolane - T

FIG. 1a

FIG. 1b

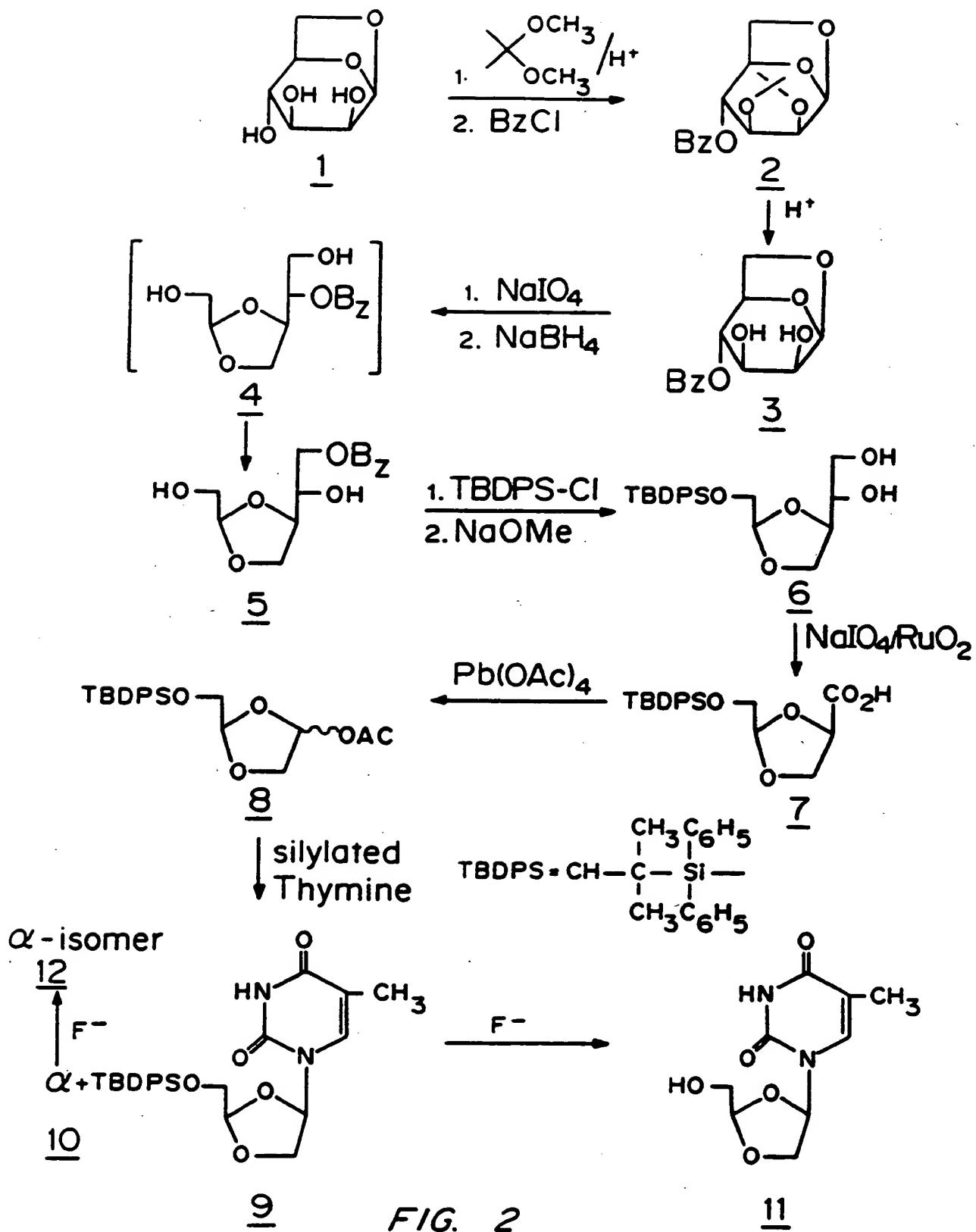
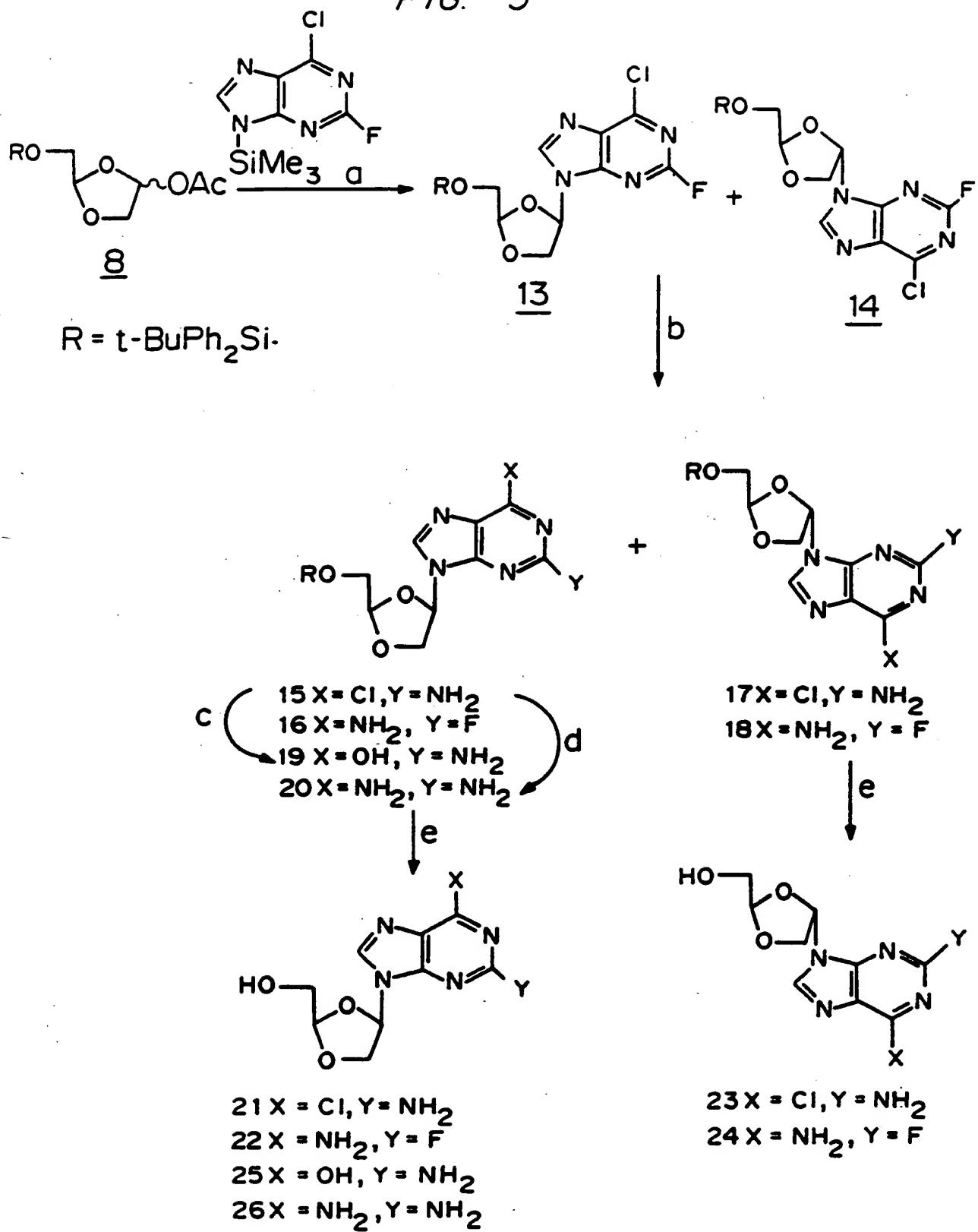


FIG. 2

FIG. 3



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US93/08044

A. CLASSIFICATION OF SUBJECT MATTER

IPC(S) : A61K 31/52; C07D 405/04, 473/00; C07F 5/02

US CL : 544/244, 264, 265, 276, 277; 514/265, 266, 885

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 544/244, 264, 265, 276, 277; 514/265, 266, 885

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS ONLINE structure search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US, A, 5,041,449 (Belleau et al) 20 August 1991, see col. 3-4, 7-8 and 9-10 and examples 12-18.	1-6 7-9
A	US, A, 5,047,407 (Belleau et al) 10 September 1991.	1-9
Y	WO, A, 90/12023 (Walker et al) 18 October 1990, see claims 1 and 25-30.	1-9
P,Y	EP, A, 0,515,156 (Mansour et al) 25 November 1992, see entire document.	1-9



Further documents are listed in the continuation of Box C.



See patent family annex.

• Special categories of cited documents:	T	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
• "A" document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
• "B" earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
• "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reasons (as specified)	"Z"	document member of the same patent family
• "O" document referring to an oral disclosure, use, exhibition or other means		
• "P" document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

01 NOVEMBER 1993

Date of mailing of the international search report

NOV 26 1993

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

Authorized officer

CECILIA TSANG

Telephone No. (703) 308-1235

Facsimile No. NOT APPLICABLE

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US93/08044

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP, A, 0,337,713 (Belleau et al) 18 October 1989, see entire document.	1-9
A,P	WO, A, 92/15308 (Painter et al) 17 September 1992.	1-9
T	US, A, 5,204,466 (Liotta et al) 20 April 1993.	1-9

PCT INTERNATIONAL APPLIC

IN TRANSMITTAL LETTER

REGARDING THE INTERNATIONAL APPLICATION OF

University of Georgia Research Foundation, Inc.

3
DOL

25 August 1993

FOR REFERENCE NUMBER

UGA390CIP

ENTITLED

ENANTIOMERICALLY PURE B-D-DIOXOLANE-NUCLEOSIDES

Certification under 37 CFR 1.10 (if applicable)

TB276407065US

"Express Mail" mailing number

25 August 1993

Date of Deposit

I hereby certify that this application is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to the Commissioner of Patents and Trademarks, Washington, D.C. 20231.

Cheryl K. Zalesky

(Typed or printed name of person
mailing application)

(Signature of person mailing
application)

To the United States Receiving Office (RO/US):

Accompanying this transmittal letter is the above-identified International application, including a completed Request form (PCT/RO/101). Please process the application according to the provisions of the Patent Cooperation Treaty.

The following requests are made of the RO/US:

1. **PREPARATION AND TRANSMITTAL OF CERTIFIED COPY OF PRIORITY DOCUMENTS**—Please prepare and transmit to the International Bureau a certified copy of the United States origin priority documents identified in Box VI of the Request form (37 CFR 1.451).

To cover the cost of copy preparation and certification (37 CFR 1.19(a)(3) and (b)(1)).

a (check) (money order) in the amount of \$ 2034.00 is attached to this transmittal letter.

the RO/US is hereby authorized to charge the following deposit account no.: _____

The appropriate Search fee for the above-named Authority is indicated on the Fee Calculation Sheet (PCT/RO/101 Annex).

2. **SUPPLEMENTAL SEARCH FEES (ONLY WHEN ISA/US CONDUCTS THE INTERNATIONAL SEARCH.)**—Please charge any Supplemental Search fees that may be required by the United States International Searching Authority (ISA/US) to deposit account no.: 11-0855

I understand that this authorization is subject to my oral confirmation thereof in each instance and that it in no way limits my right to submit a protest against payment of the Supplemental Search fees, but is merely an administrative aid to assure that the ISA/US may timely complete the Search Report.

NOTE: SUPPLEMENTAL SEARCH FEES FOR ISA/EP ARE PAYABLE DIRECTLY TO THE EUROPEAN PATENT OFFICE

3. **DISCLOSURE INFORMATION**—In order to assist in screening the accompanying International application for purposes of determining whether a license for foreign transmittal should and could be granted, the following information is supplied:

A. There is no prior filed application relating to this invention.

B. There is a prior application*, serial number 07/935,515 filed on 25/08/92 which contains subject matter that is

1. substantially identical to that of the accompanying International application.

2. less than that of the accompanying International application. The additional subject matter of the International application appears on page(s) and line(s) _____.

3. more than that of the accompanying International application.

C. Disclosure information cannot be covered by the language of Points 4A or 4B above due to the involvement of several prior applications or for other reasons. A separate sheet on which the disclosure information is explained is attached to this transmittal letter.

4. **REQUEST FOR FOREIGN TRANSMITTAL LICENSE**—According to the provisions of 35 U.S.C. 184 and 37 CFR 5.11, a license to transmit the accompanying International application to foreign agencies or international authorities is hereby requested.

* Priority is not claimed, unless all necessary information is listed in Box VI of the Request Form (PCT/RO/101).

SIGNER IS THE

APPLICANT

COMMON REPRESENTATIVE

ATTORNEY/AGENT

REG. NO. 33,052

NAME OF SIGNER (typed)

Cheryl K. Zalesky

SIGNATURE

PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

For receiving Office use only

International Application No.

International Filing Date

Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference
(if desired) (12 characters maximum) **UGA390CIP**

Box No. I TITLE OF INVENTION

ENANTIOMERICALLY PURE β -D-DIOXOLANE-NUCLEOSIDES

Box No. II APPLICANT

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)

UNIVERSITY OF GEORGIA RESEARCH FOUNDATION, INC.
The University of Georgia
Athens, Georgia 30602
U.S.A.

This person is also inventor.

Telephone No.
(706) 542-5929

Faximile No.
(706) 542-5638

Teleprinter No.

State (i.e. country) of nationality:
U.S.A.

State (i.e. country) of residence:
U.S.A.

This person is applicant for the purposes of: all designated States all designated States except the United States of America the United States of America only the States indicated in the Supplemental Box

Box No. III FURTHER APPLICANTS AND/OR (FURTHER) INVENTORS

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)

EMORY UNIVERSITY
1380 S. Oxford Road
Atlanta, Georgia 30322
U.S.A.

This person is:

applicant only

applicant and inventor

inventor only (If this check-box is marked, do not fill in below.)

State (i.e. country) of nationality:
U.S.A.

State (i.e. country) of residence:
U.S.A.

This person is applicant for the purposes of: all designated States all designated States except the United States of America the United States of America only the States indicated in the Supplemental Box

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)

CHU, CHUNG K.
120 Orchard Knob Lane
Athens, Georgia 30605
U.S.A.

This person is:

applicant only

applicant and inventor

inventor only (If this check-box is marked, do not fill in below.)

State (i.e. country) of nationality:

State (i.e. country) of residence:

This person is applicant for the purposes of: all designated States all designated States except the United States of America the United States of America only the States indicated in the Supplemental Box

Further applicants and/or (further) inventors are indicated on a continuation sheet.

Continuation of Box No. III FURTHER APPLICANTS AND/OR (FURTHER) INVENTORS

*If none of the following sub-boxes is used, this sheet is not to be included in the request.*Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

SCHINAZI, RAYMOND F.
1524 Regency Walk Drive
Decatur, Georgia 30033
U.S.A.

This person is:

- applicant only
- applicant and inventor
- inventor only *(If this check-box is marked, do not fill in below.)*

State (i.e. country) of nationality:

State (i.e. country) of residence:

This person is applicant for the purposes of:

 all designated States all designated States except the United States of America the United States of America only the States indicated in the Supplemental Box

Name and address:

(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)

This person is:

- applicant only
- applicant and inventor
- inventor only *(If this check-box is marked, do not fill in below.)*

State (i.e. country) of nationality:

State (i.e. country) of residence:

This person is applicant for the purposes of:

 all designated States all designated States except the United States of America the United States of America only the States indicated in the Supplemental Box

Name and address:

(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)

This person is:

- applicant only
- applicant and inventor
- inventor only *(If this check-box is marked, do not fill in below.)*

State (i.e. country) of nationality:

State (i.e. country) of residence:

This person is applicant for the purposes of:

 all designated States all designated States except the United States of America the United States of America only the States indicated in the Supplemental Box

Name and address:

(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)

This person is:

- applicant only
- applicant and inventor
- inventor only *(If this check-box is marked, do not fill in below.)*

State (i.e. country) of nationality:

State (i.e. country) of residence:

This person is applicant for the purposes of:

 all designated States all designated States except the United States of America the United States of America only the States indicated in the Supplemental Box Further applicants and/or (further) inventors are indicated on another continuation sheet.

Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as:

 agent common representative

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.) ZALESKY, Cheryl K.; PRATT, John S.; EWING, IV, James L.; PABST, Patrea L.; ROSELL, Dean W. KILPATRICK & CODY, 1100 Peachtree Street, Suite 2800 Atlanta, Georgia 30309	Telephone No. (404) 815-6439
	Fax/telex No. (404) 815-6555
	Telex/teletype No.

Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Box No. V DESIGNATION OF STATES

The following designations are hereby made under Rule 4.9(a) (mark the applicable check-boxes; at least one must be marked):

Regional Patent

- EP European Patent AT Austria, BE Belgium, CH and LI Switzerland and Liechtenstein, DE Germany, DK Denmark, ES Spain, FR France, GB United Kingdom, GR Greece, IE Ireland, IT Italy, LU Luxembourg, MC Monaco, NL Netherlands, PT Portugal, SE Sweden, and any other State which is a Contracting State of the European Patent Convention and of the PCT
- OA OAPI Patent: Benin, Burkina Faso, Cameroon, Central African Republic, Chad, Congo, Côte d'Ivoire, Gabon, Guinea, Mali, Mauritania, Niger, Senegal, Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT (if other kind of protection or treatment desired, specify on dotted line)

National Patent (if other kind of protection or treatment desired, specify on dotted line):

- | | |
|---|--|
| <input type="checkbox"/> AT Austria | <input type="checkbox"/> MN Mongolia |
| <input checked="" type="checkbox"/> AU Australia | <input type="checkbox"/> MW Malawi |
| <input type="checkbox"/> BB Barbados | <input type="checkbox"/> NL Netherlands |
| <input type="checkbox"/> BG Bulgaria | <input type="checkbox"/> NO Norway |
| <input type="checkbox"/> BR Brazil | <input type="checkbox"/> NZ New Zealand |
| <input type="checkbox"/> BY Belarus | <input type="checkbox"/> PL Poland |
| <input checked="" type="checkbox"/> CA Canada | <input type="checkbox"/> PT Portugal |
| <input type="checkbox"/> CH and LI Switzerland and Liechtenstein | <input type="checkbox"/> RO Romania |
| <input type="checkbox"/> CZ Czech Republic | <input type="checkbox"/> RU Russian Federation |
| <input type="checkbox"/> DE Germany | <input type="checkbox"/> SD Sudan |
| <input type="checkbox"/> DK Denmark | <input type="checkbox"/> SE Sweden |
| <input type="checkbox"/> ES Spain | <input type="checkbox"/> SK Slovakia |
| <input type="checkbox"/> FI Finland | <input type="checkbox"/> UA Ukraine |
| <input type="checkbox"/> GB United Kingdom | <input type="checkbox"/> US United States of America |
| <input type="checkbox"/> HU Hungary | <input type="checkbox"/> VN Viet Nam |
| <input checked="" type="checkbox"/> JP Japan | |
| <input type="checkbox"/> KP Democratic People's Republic of Korea | |
| <input type="checkbox"/> KR Republic of Korea | |
| <input type="checkbox"/> KZ Kazakhstan | |
| <input type="checkbox"/> LK Sri Lanka | |
| <input type="checkbox"/> LU Luxembourg | |
| <input type="checkbox"/> MG Madagascar | |

Check-boxes reserved for designating States (for the purposes of a national patent) which have become party to the PCT after issuance of this sheet:

-
-
-
-

In addition to the designations made above, the applicant also makes under Rule 4.9(b) all designations which would be permitted under the PCT except the designation(s) of _____.

The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. (Confirmation of a designation consists of the filing of a notice specifying that designation and the payment of the designation and confirmation fees. Confirmation must reach the receiving Office within the 15-month time limit.)

Box No. VI PRIORITY CLAIM.Further priority claims are indicated in the Supplemental Box

The priority of the following earlier application(s) is hereby claimed:

Country (in which, or for which, the application was filed)	Filing Date (day/month/year)	Application No.	Office of filing (only for regional or international application)
item (1) U.S.	25/08/92	07/935,515	
item (2)			
item (3)			

Mark the following check-box if the certified copy of the earlier application is to be issued by the Office which for the purposes of the present international application is the receiving Office (a fee may be required):

The receiving Office is hereby requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) identified above as item(s) : (1)

Box No. VII EARLIER SEARCH

Fill in where a search (international, international-type or other) by the International Searching Authority has already been carried out or requested and the reference to the relevant application (or the translation thereof) or by reference to the search request:

Country (or regional Office):

Date (day/month/year):

Number:

Box No. VIII CHECK LIST

This international application contains the following number of sheets:

- | | | | |
|----------------|---|----|--------|
| 1. request | : | 4 | sheets |
| 2. description | : | 34 | sheets |
| 3. claims | : | 5 | sheets |
| 4. abstract | : | 1 | sheets |
| 5. drawings | : | 3 | sheets |
| Total | : | 47 | sheets |

This international application is accompanied by the item(s) marked below:

- | | |
|---|--|
| 1. <input type="checkbox"/> separate signed power of attorney | 5. <input checked="" type="checkbox"/> fee calculation sheet |
| 2. <input checked="" type="checkbox"/> copy of general power of attorney | 6. <input type="checkbox"/> separate indications concerning deposited microorganisms |
| 3. <input type="checkbox"/> statement explaining lack of signature | 7. <input type="checkbox"/> nucleotide and/or amino acid sequence listing (disease) |
| 4. <input type="checkbox"/> priority document(s) identified in Box No. VI as item(s): | 8. <input checked="" type="checkbox"/> other (specify):
copy of Assignment documents |

Figure No. _____ of the drawings (if any) should accompany the abstract when it is published.

Box No. IX SIGNATURE OF APPLICANT OR AGENT

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).



 Cheryl K. Zalesky

For receiving Office use only

1. Date of actual receipt of the purported international application:	2. Drawings: <input type="checkbox"/> received: <input type="checkbox"/> not received:
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:	
4. Date of timely receipt of the required corrections under PCT Article 11(2):	
5. International Searching Authority specified by the applicant: ISA /	6. <input type="checkbox"/> Transmittal of search copy delayed until search fee is paid

For International Bureau use only

Date of receipt of the record copy
by the International Bureau: